ATTACHMENT D

QUARTERLY PROGRESS REPORT FOR THE PERIOD Apr-Jun, [redacted]

PROJECT TITLE: CounterBW Point Detectors DOE/HQ PMIS)
DOE/HQ PROJECT NUMBER: CB04LL LAB/CONTRACTOR: LLNL
B&R CODE: GC0404 DATE: [redacted]
PRINCIPAL INVESTIGATOR(S): Dr. R. Mariella Jr., LLNL, 925-422-8905
HQ PROJECT MANAGER: (Page Stoutland, NN- 1, (202) 586-3263)

PROGRESS DURING THIS QUARTER:
Les Jones and Kodumudi Venkateswaran from LLNL visited Mark Kingsley and Gary Dennis at PNNL from May 26 to May 28. The purpose of the visit was to test the version-1 LLNL autonomous pathogen detection system in simulated field conditions over an extended period of operation. The autonomous detector consists of a Research International SASS2000 aerosol collector coupled with a custom fluidic system built at LLNL using many off-the-shelf components coupled with a Microcyte flow cytometer. The entire system is normally rack mounted. A central computer runs instructs the aerosol collector to collect sample, the sample preparation to meter and mix the sample with reagents consisting primarily of antibody coated beads, and the flow cytometer to check for binding of the antigen to the antibodies. The PNNL facility consists of an enclosed wind tunnel with three methods of injection of particles into the flow stream. The three methods are fluidized bed, and nebulization in sugar or in methane. PNNL has a system that can be used to measure the size and concentration of the aerosol particles. *B.g.* was the simulant used to test the system.

The LLNL system was installed at PNNL on May 26. On May 27, a 10-hour run was performed followed by another 2 hour run. Samples were collected every 15 minutes during these runs. A 6-hour run was conducted on May 28 prior to the system being shipped back to LLNL. In the course of these runs, all three methods for dispersion of B.g. were tested. The system behaved well during the entire test period. The system routinely called positives down to the 300 ACPLA level, including aerosols in which the particles were actually monodispersed spores, not clumps. The major improvement to increase sensitivity will be to replace the Microcyte flow cytometer with the Luminex flow cytometer. This is scheduled to be a part of the version 2 system.

We have performed evaluations of the hybrid aerosol collector from Research International, Inc., that combines the latest version of their wetted-wall cyclone collector with the LLNL-designed virtual impactor. The collection efficiency for the 1 to 10-μm particles remains the same in terms of extraction from the atmosphere, and the hybrid unit samples four times larger volume of air per

minute than the original RI collector. We are considering further refinements to increase the utility of this system.

COMMENTS:

The autonomous instrument performed as well at the wind tunnel as it did at LLNL, which is really good news. The flow-cytometric, antibody-based single-target assay for B.g. was implemented on the very simple Microcyte flow cytometer, which is about 1000X lower performance than the miniFlo we took to JFT III, and about 100X lower performance than the Luminex flow cytometer we are now working to integrate into the Autonomous Pathogen Detection System. The system reliably detected aerosols of 300 spores/liter of air (not clumped particles consisting of 15 spores per particle). Assuming that future challenges will be of the Dugway variety with "optimized" clumps/particles of roughly 15 spores/particle and assuming that we will be using the Luminex instead of the Microcyte, 1 ACPLA still looks like an achievable level of performance.

FUNDING STATUS:

	OPER$	CAP $
UNCOSTED FROM PREVIOUS FY:		
CURRENT FYFUNDING:		
TOTAL FUNDINGAVAILABLE:	$K	$0K
$ SPENT THISQUARTER:	$K	$0K
$ SPENT YEAR-TO-DATE:	$K	$0K
$ REMAINING FORTHIS FY:	$K	$0K
ANTICIPATED UNCOSTED CURRENT FY FUNDS:	$K	$0K

TECHNICAL REPORTS/PRESENTATIONS TO:

Phillip Belgrader, William Benett, Dean Hadley, James Richards, Paul Stratton, Raymond Mariella Jr., and Fred Milanovich, "PCR analysis of bacteria in 7 minutes", *Science*, **284**, 449 ([redacted]

3 June 1999 Demonstration of the APDS to Senators J.James Exon, Anthony C. Beilson, and Suzanne E. Spaulding, and others from the Commission to Assess the Organization of the Federal Government to Combat the Proliferation of WMD.

[redacted] Demonstration of the APDS to Lloyd Salvetti Director, Center for the Study of Intelligence, Central Intelligence Agency (and co-workers).